CLAIMS

1. An antibody or a functional fragment thereof, binding to TRAIL-R1 and/or TRAIL-R2.

2. The antibody or the functional fragment thereof of claim 1, having at least one property selected from the following (a) to (c) of:

(a) having activity to induce apoptosis in carcinoma cells expressing TRAIL-R1 and/or TRAIL-R2;

(b) not having effect on normal human cells expressing TRAIL-R1 and/or TRAIL-R2; and

(c) not inducing human hepatocyte toxicity.

3. An antibody or a functional fragment thereof, having all the following properties (a) to (c) of:

(a) having activity to induce apoptosis in carcinoma cells expressing TRAIL-R1 and/or TRAIL-R2;

(b) not having effect on normal human cells expressing TRAIL-R1 and/or TRAIL-R2; and

(c) not inducing human hepatocyte toxicity.

4. The antibody or the functional fragment thereof of claim 2 or 3, which binds to TRAIL-R2, but does not bind to TRAIL-R1.

5. The antibody or the functional fragment thereof of claim 2 or 3, which binds to both TRAIL-R2 and TRAIL-R1.

6. The antibody or the functional fragment thereof of any one of claims 1 to 5, which is a monoclonal antibody produced by a mouse-mouse hybridoma.

7. The antibody or the functional fragment thereof of any one of claims 1 to 6, which is a human antibody.

8. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 0.01 μg/ml or more for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours.

9. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 0.1 μg/ml or more for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours.

10. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 2 to 10 μg/ml for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours.

11. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 10 μg/ml or more for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours.

12. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 10 to 100 μg/ml for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours.

13. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 100 μg/ml or more for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours.

14. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 100 μg/ml or less for carcinoma cells when the number of cells is 2.5×10^3 and the reaction time is 48 hours.

15. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 10 μg/ml or less for carcinoma cells when the number of cells is 2.5×10^3 and the reaction time is 48 hours.

16. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 0.7 μg/ml or less for carcinoma cells when the number of cells is 2.5×10^3 and the reaction time is 48 hours.

17. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 0.02 to 0.11 μg/ml for carcinoma cells when the number of cells is 2.5×10^3 and the reaction time is 48 hours.

18. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 0.02 μg/ml or less for carcinoma cells when the number

of cells is 2.5×10^3 and the reaction time is 48 hours.

19. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 2 to 100 μg/ml for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours, and having an LD50 value of 0.02 to 0.11 μg/ml for carcinoma cells when the number of cells is 2.5×10^3 and the reaction time is 48 hours.

20. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours is 2 times or more greater than the LD50 value for carcinoma cells when the number of cells is 2.5×10^3 and the reaction time is 48 hours.

21. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours is 10 times or more greater than the LD50 value for carcinoma cells when the number of cells is 2.5×10^3 and the reaction time is 48 hours.

22. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours is 50 times or more greater than the LD50 value for carcinoma cells when the number of cells is 2.5×10^3 and the reaction time is 48 hours.

23. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours is 50 to 100 times greater than the LD50 value for carcinoma cells when the number of cells is 2.5×10^3 and the reaction time is 48 hours.

24. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours is 100 times or more greater than the

LD50 value for carcinoma cells when the number of cells is 2.5×10^3 and the reaction time is 48 hours.

25. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours is 100 to 1000 times greater than the LD50 value for carcinoma cells when the number of cells is 2.5×10^3 and the reaction time is 48 hours.

26. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours is 250 to 1000 times greater than the LD50 value for carcinoma cells when the number of cells is 2.5×10^3 and the reaction time is 48 hours.

27. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is 7.5×10^4 and the reaction time is 24 hours is 1000 times or more greater than the LD50 value for carcinoma cells when the number of cells is 2.5×10^3 and the reaction time is 48 hours.

28. The antibody or the functional fragment thereof of any one of claims 8 to 27, wherein a reaction volume is between 110 and 120 μl.

29. The antibody or the functional fragment thereof of any one of claims 2, 3 and 14 to 28, wherein the carcinoma cells are Colo205 cells.

30. The antibody or the functional fragment thereof of claim 2 or 3, wherein the carcinoma cells are Colo205 cells, U251 cells or Jurkat cells.

31. The antibody or the functional fragment thereof of any one of claims 1 to 30, which can suppress tumor growth or regress tumors.

32. The antibody or the functional fragment thereof of claim 31, wherein the tumor is at least one tumor selected from the group consisting of colon cancer, colorectal cancer, lung cancer, breast cancer, brain tumor, malignant melanoma, renal cell carcinoma, bladder cancer, leukemia, lymphomas, T cell lymphomas,

multiple myeloma, gastric cancer, pancreas cancer, cervical cancer, endometrial carcinoma, ovarian cancer, esophageal cancer, liver cancer, head and neck squamous cell carcinoma, cutaneous cancer, urinary tract carcinoma, prostate cancer, choriocarcinoma, pharyngeal cancer, laryngeal cancer, thecomatosis, androblastoma, endometrium hyperplasy, endometriosis, embryoma, fibrosarcoma, Kaposi's sarcoma, hemangioma, cavernous hemangioma, angioblastoma, retinoblastoma, astrocytoma, neurofibroma, oligodendroglioma, medulloblastoma, ganglioneuroblastoma, glioma, rhabdomyosarcoma, hamartoblastoma, osteogenic sarcoma, leiomyosarcoma, thyroid sarcoma, Wilms tumor and the like.

33. The antibody or the functional fragment thereof of claim 31, wherein the tumor is derived from Colo205 cells transplanted in a nude mouse.

34. The antibody or the functional fragment thereof of any one of claims 31 to 33, wherein a period during which tumor growth can be suppressed or tumor regression can be achieved is at least 9 days.

35. The antibody or the functional fragment thereof of any one of claims 31 to 34, wherein the dose of the antibody or the functional fragment thereof is 100 μg/body or 5 mg/kg.

36. The antibody or the functional fragment thereof of any one of claims 31 to 34, wherein the dose of the antibody or the functional fragment thereof is 20 μg/body or 1 mg/kg.

37. The antibody or the functional fragment thereof of any one of claims 31 to 34, wherein the dose of the antibody or the functional fragment thereof is 4 μg/body or 200μg/kg.

38. The antibody or the functional fragment thereof of any one of claims 31 to 34, wherein the dose of the antibody or the functional fragment thereof is 1 μg/body or 50 μg/kg.

39. The antibody or the functional fragment thereof of any one of claims 1 to 38, which is an immunoglobulin G antibody.

40. An antibody or a functional fragment thereof, which can induce an average of 14% or more tumor reduction by 4 days after the initial administration, when administered at a concentration of 20 μg/mouse to a 4- to 6-week-old tumor-bearing mouse having a 100 mm^3 tumor.

41. The antibody or the functional fragment thereof of claim 40, which can maintain an average of 14% or more tumor reduction for at least 7 days.

42. The antibody or the functional fragment thereof of claim 40, which can induce an average of 65% or more tumor reduction by 4 days after the initial administration, when administered at a concentration of 20 μg/mouse to a 4- to 6-week-old tumor-bearing mouse having a 100 mm^3 tumor.

43. The antibody or the functional fragment thereof of claim 40, which can induce an average of 80% or more tumor reduction by 7 days after the initial administration, when administered at a concentration of 20 μg/mouse to a 4- to 6-week-old tumor-bearing mouse having a 100 mm^3 tumor.

44. The antibody or the functional fragment thereof of claim 43, which can maintain an average of 80% or more tumor reduction for at least 4 days.

45. The antibody or the functional fragment thereof of claim 40, which can induce an average of 45% or more tumor reduction by 3 days after the initial administration, when administered at a concentration of 25 μg/mouse to a 12-week-old tumor-bearing mouse having a 100 mm^3 tumor.

46. The antibody or the functional fragment thereof of claim 45, which can induce an average of 65% or more tumor reduction by 5 days after the initial administration, when administered at a concentration of 25 μg/mouse to a 12-week-old tumor-bearing mouse having a 100 mm^3 tumor.

47. The antibody or the functional fragment thereof of claim 46, which can maintain an average of 65% or more tumor reduction for at least 27 days.

48. The antibody or the functional fragment thereof of claim 40, which can induce an average of 39% or more tumor reduction by 4 days after the initial administration, when administered at a concentration of 20 μg/mouse to a 4- to

6-week-old tumor-bearing mouse having a 300 mm^3 tumor.

49. The antibody or the functional fragment thereof of claim 48, which can maintain an average of 39% or more tumor reduction for at least 14 days.

50. The antibody or the functional fragment thereof of claim 40, which is a 0304 antibody.

51. The antibody or the functional fragment thereof of claim 40, which is an E-11-13 antibody.

52. An antibody or a functional fragment thereof binding to TRAIL-R1 and/or TRAIL-R2, which is produced by a hybridoma E-11-13, H-48-2, L-30-10, N-18-12, W-40-5, X-14-4, X-51-12, F-4-8, G-3-10, 0304 or KMTR1.

53. An antibody or a functional fragment thereof binding to TRAIL-R1 and/or TRAIL-R2, which is produced by a hybridoma H-48-2 with the accession number of FERM BP-7599, a hybridoma E-11-13 with the accession number of FERM BP-7698 or FERM BP-7770, a hybridoma F-4-8 with the accession number of FERM BP-7699 or FERM BP-7768, a hybridoma L-30-10 with the accession number of FERM BP-7700 or FERM BP-7769, a hybridoma 0304 with the accession number of FERM BP-8037, or a hybridoma KMTR1 with the accession number of FERM BP-8038.

54. An antibody or a functional fragment thereof, having amino acid sequences of the mature portions of a heavy chain variable region and a light chain variable region of the antibody produced by a hybridoma E-11-13, which are respectively represented by SEQ ID NOS: 17 and 19; a heavy chain variable region and a light chain variable region of the antibody produced by a hybridoma L-30-10, which are respectively represented by SEQ ID NOS: 21 and 23; a heavy chain variable region and a light chain variable region of the antibody produced by a hybridoma H-48-2, which are respectively represented by SEQ ID NOS: 25 and 27; a heavy chain variable region and a light chain variable region of the antibody produced by a hybridoma 0304, which are respectively represented by SEQ ID NOS: 29 and 31; or a heavy chain variable region and a light chain variable region of the

antibody produced by a hybridoma KMTR1, which are respectively represented by SEQ ID NOS: 33 and 35.

55. An antibody or a functional fragment thereof, having amino acid sequences of the mature portions of a heavy chain variable region and a light chain variable region that are encoded by nucleic acid sequences isolated from a hybridoma E-11-13, which are respectively represented by SEQ ID NOS: 16 and 18; a heavy chain variable region and a light chain variable region that are encoded by nucleic acid sequences isolated from a hybridoma L-30-10, which are respectively represented by SEQ ID NOS: 20 and 22; a heavy chain variable region and a light chain variable region that are encoded by nucleic acid sequences isolated from a hybridoma H-48-2, which are respectively represented by SEQ ID NOS: 24 and 26; a heavy chain variable region and a light chain variable region that are encoded by nucleic acid sequences isolated from a hybridoma 0304, which are respectively represented by SEQ ID NOS: 28 and 30; or a heavy chain variable region and a light chain variable region that are encoded by nucleic acid sequences isolated from a hybridoma KMTR1, which are respectively represented by SEQ ID NOS: 32 and 34.

56. A hybridoma producing monoclonal antibodies that bind to TRAIL-R2, which is selected from the group consisting of E-11-13, H-48-2, L-30-10, N-18-12, W-40-5, X-14-4, X-51-12, F-4-8, G-3-10, 0304 and KMTR1.

57. A hybridoma producing monoclonal antibodies that bind to TRAIL-R2, which is selected from a hybridoma H-48-2 with the accession number of FERM BP-7599, a hybridoma E-11-13 with the accession number of FERM BP-7698 or FERM BP-7770, a hybridoma F-4-8 with the accession number of FERM BP-7699 or FERM BP-7768, a hybridoma L-30-10 with the accession number of FERM BP-7700 or FERM BP-7769, a hybridoma 0304 with the accession number of FERM BP-8037 and a hybridoma KMTR1 with the accession number of FERM BP-8038.

58. A method for producing anti-TRAIL-R2 monoclonal antibodies, comprising

culturing the hybridoma of claim 56 or 57, and collecting the antibodies binding to TRAIL-R2 from the obtained culture product.

59. A method for producing anti-TRAIL-R2 monoclonal antibodies, comprising isolating a gene encoding an anti-TRAIL-R2 monoclonal antibody from the hybridoma of claim 56 or 57, constructing an expression vector having the gene, introducing the expression vector into a host to express the monoclonal antibody, and collecting anti-TRAIL-R2 monoclonal antibodies from the obtained host, or the culture supernatant or the secretion of the host.

60. The production method of claim 59, wherein the host is any host selected from the group consisting of *Escherichia coli*, yeast cells, insect cells, mammalian cells and plant cells, and mammals.

61. A prophylactic or therapeutic agent against tumors, comprising as an active ingredient the antibody or the functional fragment thereof of any one of claims 1 to 55.

62. The prophylactic or therapeutic agent of claim 61, wherein the tumor is any one tumor selected from the group consisting of colon cancer, colorectal cancer, lung cancer, breast cancer, brain tumor, malignant melanoma, renal cell carcinoma, bladder cancer, leukemia, lymphomas, T cell lymphomas, multiple myeloma, gastric cancer, pancreas cancer, cervical cancer, endometrial carcinoma, ovarian cancer, esophageal cancer, liver cancer, head and neck squamous cell carcinoma, cutaneous cancer, urinary tract carcinoma, prostate cancer, choriocarcinoma, pharyngeal cancer, laryngeal cancer, thecomatosis, androblastoma, endometrium hyperplasy, endometriosis, embryoma, fibrosarcoma, Kaposi's sarcoma, hemangioma, cavernous hemangioma, angioblastoma, retinoblastoma, astrocytoma, neurofibroma, oligodendroglioma, medulloblastoma, ganglioneuroblastoma, glioma, rhabdomyosarcoma, hamartoblastoma, osteogenic sarcoma, leiomyosarcoma, thyroid sarcoma, Wilms tumor and the like.

63. An antibody or a functional fragment thereof of any one of claims 1 to 49,

which binds to TRAIL-R and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors.

64. An antibody or a functional fragment thereof of any one of claims 1 to 49, which binds to TRAIL-R and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells in the following test using the said antibody or functional fragment thereof is 80% or less,

(1) Preparing Colo205 cells (ATCC No.CCL-222) which were colon carcinoma cells, at a concentration of 1.0 x 10^5/ml in RPMI-1640 medium containing 10% FCS, adding the cells to each well of a 96-well flat-bottomed plate at 100μl/well and culturing at 37°C under 5.0% carbon dioxide gas for 24 hours,

(2) Adding to each well of (1) an antibody or a functional fragment thereof which is bound to TRAIL-R dissolved in RPMI-1640 medium containing 10% FCS such that a concentration of the antibody or the functional fragment thereof becomes 1000ng/ml when it is added to each well at 10μl/well, culturing each well at 37°C under 5.0% carbon dioxide gas for 48 hours, washing each well once with PBS and adding a fresh RPMI-1640 medium containing 10% FCS at100μl/well,

(3) Adding 20 μl of MTS reagent (Cell Titer 96® AQUEOUS Non-Radioactive Cell Proliferation Assay: Promega) to each well of (2) and culturing at 37°C under 5.0% carbon dioxide gas for 2 hours, and

(4) Measuring absorbance of each well of (3) at a wavelength of 490 nm (with a reference wavelength of 630 nm) using a microplate reader and calculating the survival rate of the cells using the reducibility of the mitochondria as an indicator,

wherein the survival rate of the cells is calculates using the following formula,

Survival rate (%) = 100 × (a-b)/(c-b) (wherein "a" represents the measured value of a well tested, "b" represents the measured value of a carcinomacell-free well, and "c" represents (i) the measured value of a well containing carcinomacells and a control antibody which is not bound to carcinoma cells and has the same

subclass with the antibody or the functional fragment thereof bound to TRAIL-R when the antibody or the functional fragment thereof has a constant region, or (ii) the measured value of a well containing carcinoma cells and a control antibody which is not bound to the carcinoma cells and does not have a constant region when the antibody or the functional fragment thereof does not have a constant region).

65. An antibody or a functional fragment thereof of claim 64, wherein the survival rate is 60% or less.

66. An antibody or a functional fragment thereof of claim 64, wherein the survival rate is 40% or less.

67. An antibody or a functional fragment thereof of claim 64, wherein the survival rate is 20% or less.

68. An antibody or a functional fragment thereof of claim 64, wherein the survival rate is 10% or less.

69. An antibody of any one of claims 1 to 49, which binds to TRAIL-R and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells in the following test using the said antibody is 80% or less,

(1) Preparing Colo205 cells (ATCC No.CCL-222) which were colon carcinoma cells, at a concentration of 1.0×10^5/ml in RPMI-1640 medium containing 10% FCS, adding the cells to each well of a 96-well flat-bottomed plate at 100μl/well and culturing at 37°C under 5.0% carbon dioxide gas for 24 hours,

(2) Adding to each well of (1) an antibody which is bound to TRAIL-R dissolved in RPMI-1640 medium containing 10% FCS such that a concentration of the antibody becomes 1000ng/ml when it is added to each well at 10μl/well, culturing each well at 37°C under 5.0% carbon dioxide gas for 48 hours, washing each well once with PBS and adding a fresh RPMI-1640 medium containing 10% FCS at 100μl/well,

(3) Adding 20 μl of MTS reagent (Cell Titer 96® AQ$_{UEOUS}$ Non-Radioactive Cell

Proliferation Assay: Promega) to each well of (2) and culturing at 37°C under 5.0% carbon dioxide gas for 2 hours, and

(4) Measuring absorbance of each well of (3) at a wavelength of 490 nm (with a reference wavelength of 630 nm) using a microplate reader and calculating the survival rate of the cells using the reducibility of the mitochondria as an indicator,

wherein the survival rate of the cells is calculates using the following formula,

Survival rate (%) = 100 × (a-b)/(c-b) (wherein "a" represents the measured value of a well tested, "b" represents the measured value of a carcinomacell-free well, and "c" represents the measured value of a well containing carcinoma cells and a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to the carcinoma cells).

70. An antibody of claim 69, wherein the survival rate is 60% or less.

71. An antibody of claim 69, wherein the survival rate is 40% or less.

72. An antibody of claim 69, wherein the survival rate is 20% or less.

73. An antibody of claim 69, wherein the survival rate is 10% or less.

74. An antibody of any one of claims 1 to 49, which binds to TRAIL-R and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells in the following test using the said antibody is 80% or less,

(1) Preparing Colo205 cells (ATCC No.CCL-222) which were colon carcinoma cells, at a concentration of 5 x 10^4/ml in RPMI-1640 medium containing 10% FCS, adding the cells to each well of a 96-well flat-bottomed plate at 100μl/well and culturing at 37°C under 5.0% carbon dioxide gas for 24 hours,

(2) Adding to each well of (1) an antibody which is bound to TRAIL-R dissolved in RPMI-1640 medium containing 10% FCS such that a concentration of the antibody becomes 1000ng/ml when it is added to each well at 10μl/well, culturing at 37°C under 5.0% carbon dioxide gas for 1 hour, adding a control antibody which has the same subclass with the antibody bound to TRAIL-R and

is not bound to carcinoma cells such that a concentration is 100μg/ml, adding goat anti-human IgG (γ)-specific polyclonal antibodies at a final concentration of 10μg/ml, culturing each well at 37°C under 5.0% carbon dioxide gas for 2 days, washing each well once with PBS and adding a fresh RPMI-1640 medium containing 10% FCS at 100μl/well,

(3) Adding 20 μl of MTS reagent (Cell Titer 96® AQUEOUS Non-Radioactive Cell Proliferation Assay: Promega) to each well of (2) and culturing at 37°C under 5.0% carbon dioxide gas for 2 hours, and

(4) Measuring absorbance of each well of (3) at a wavelength of 490 nm (with a reference wavelength of 630 nm) using a microplate reader and calculating the survival rate of the cells using the reducibility of the mitochondria as an indicator,

wherein the survival rate of the cells is calculates using the following formula,

Survival rate (%) = 100 × (a-b)/(c-b) (wherein "a" represents the measured value of a well tested, "b" represents the measured value of a carcinoma cell-free well, and "c" represents the measured value of a well containing carcinoma cells and a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to the carcinoma cells).

75. An antibody of claim 74, wherein the survival rate is 60% or less.

76. An antibody of claim 74, wherein the survival rate is 40% or less.

77. An antibody of claim 74, wherein the survival rate is 20% or less.

78. An antibody of claim 74, wherein the survival rate is 10% or less.

79. An antibody of any one of claims 1 to 49, which binds to TRAIL-R and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells in the following test using the said antibody is 80% or less,

(1) Preparing Colo205 cells (ATCC No.CCL-222) which were colon carcinoma cells, at a concentration of 5 x 10^4/ml in RPMI-1640 medium containing 10% FCS, adding the cells to each well of a 96-well flat-bottomed plate at 100μl/well

and culturing at 37°C under 5.0% carbon dioxide gas for 24 hours,

(2) Adding to each well of (1) an antibody which is bound to TRAIL-R dissolved in RPMI-1640 medium containing 10% FCS such that a concentration of the antibody becomes 1000ng/ml when it is added to each well at 10μl/well, culturing at 37°C under 5.0% carbon dioxide gas for 1 hour, adding a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to carcinoma cell such that a concentration is 3μg/ml, adding goat anti-human IgG (γ)-specific polyclonal antibodies at a final concentration of 10μg/ml, culturing each well at 37°C under 5.0% carbon dioxide gas for 2 days, washing each well once with PBS and adding a fresh RPMI-1640 medium containing 10% FCS at 100μl/well,

(3) Adding 20 μl of MTS reagent (Cell Titer 96® AQUEOUS Non-Radioactive Cell Proliferation Assay: Promega) to each well of (2) and culturing at 37°C under 5.0% carbon dioxide gas for 2 hours, and

(4) Measuring absorbance of each well of (3) at a wavelength of 490 nm (with a reference wavelength of 630 nm) using a microplate reader and calculating the survival rate of the cells using the reducibility of the mitochondria as an indicator,

wherein the survival rate of the cells is calculates using the following formula,

Survival rate (%) = 100 × (a-b)/(c-b) (wherein "a" represents the measured value of a well tested, "b" represents the measured value of a carcinoma cell-free well, and "c" represents the measured value of a well containing carcinoma cells and a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to the carcinoma cells).

80. An antibody of claim 79, wherein the survival rate is 60% or less.

81. An antibody of claim 79, wherein the survival rate is 40% or less.

82. An antibody of claim 79, wherein the survival rate is 20% or less.

83. An antibody of claim 79, wherein the survival rate is 10% or less.

84. An antibody or a functional fragment thereof of any one of claims 1 to 49,

which binds to TRAIL-R and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells on condition that (1)1.0 x 10^5/ml of carcinoma cells and (2)1000ng/ml of the antibody or the functional fragment thereof are cultured at 37°C under 5.0% carbon dioxide gas for 48 hours is 80% or less,

85. An antibody or a functional fragment thereof of claim 84, wherein the survival rate is 60% or less.

86. An antibody or a functional fragment thereof of claim 84, wherein the survival rate is 40% or less.

87. An antibody or a functional fragment thereof of claim 84, wherein the survival rate is 20% or less.

88. An antibody or a functional fragment thereof of claim 84, wherein the survival rate is 10% or less.

89. An antibody or a functional fragment thereof of any one of claims 1 to 49, which binds to TRAIL-R and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells on condition that (1)5 x 10^4/ml of carcinoma cells, (2)1000ng/ml of the antibody, (3)100μg/ml of a control antibody or a functional fragment thereof which has the same subclass with the antibody or the functional fragment thereof bound to TRAIL-R and is not bound to carcinoma cells and (4)an antibody which binds to both the antibody or the functional fragment thereof bound to TRAIL-R and the control antibody are cultured at 37°C under 5.0% carbon dioxide gas for 48 hours is 80% or less.

90. An antibody or a functional fragment thereof of claim 89, wherein the survival rate is 60% or less.

91. An antibody or a functional fragment thereof of claim 89, wherein the survival rate is 40% or less.

92. An antibody or a functional fragment thereof of claim 89, wherein the survival rate is 20% or less.

93. An antibody or a functional fragment thereof of claim 89, wherein the survival rate is 10% or less.

94. An antibody or a functional fragment thereof of any one of claims 1 to 49, which binds to TRAIL-R and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells on condition that (1)5 x 10^4/ml of carcinoma cells, (2)1000ng/ml of the antibody, (3)3μg/ml of a control antibody or a functional fragment thereof which has the same subclass with the antibody or the functional fragment thereof bound to TRAIL-R and is not bound to carcinoma cells and (4)an antibody which binds to both the antibody or the functional fragment thereof bound to TRAIL-R and the control antibody are cultured at 37°C under 5.0% carbon dioxide gas for 48 hours is 80% or less.

95. An antibody or a functional fragment thereof of claim 94, wherein the survival rate is 60% or less.

96. An antibody or a functional fragment thereof of claim 94, wherein the survival rate is 40% or less.

97. An antibody or a functional fragment thereof of claim 94, wherein the survival rate is 20% or less.

98 An antibody or a functional fragment thereof of claim 94, wherein the survival rate is 10% or less.

99. An antibody or a functional fragment thereof of any one of claims 84 to 98, wherein the carcinoma cell is Colo205.

100. An antibody or a functional fragment thereof which is bound to TRAIL-R of any one of claims 1 to 49, the activity to induce apoptosis of which antibody or a functional fragment thereof on carcinoma cells expressing TRAIL-R does not substantially change depending on the presence or absence of an antibody which is bound to a constant region of the said antibody which is bound to TRAIL-R.

101. An antibody or a functional fragment thereof which is bound to TRAIL-R of any one of claims 1 to 49, wherein the survival rate of carcinoma cells

expressing TRAIL-R does not substantially change depending on the presence or absence of an antibody which is bound to a constant region of the said antibody which is bound to TRAIL-R.

102. A therapeutic composition, comprising as an active ingredient the antibody or the functional fragment thereof of any one of claims 63 to 101.

103. A prophylactic or therapeutic agent against tumors, comprising as an active ingredient the antibody or the functional fragment thereof of any one of claims 63 to 101.

104. A prophylactic or therapeutic agent against tumors of claim 103, wherein the tumor is any one tumor selected from the group consisting of colon cancer, colorectal cancer, lung cancer, breast cancer, brain tumor, malignant melanoma, renal cell carcinoma, bladder cancer, leukemia, lymphomas, T cell lymphomas, multiple myeloma, gastric cancer, pancreas cancer, cervical cancer, endometrial carcinoma, ovarian cancer, esophageal cancer, liver cancer, head and neck squamous cell carcinoma, cutaneous cancer, urinary tract carcinoma, prostate cancer, choriocarcinoma, pharyngeal cancer, laryngeal cancer, thecomatosis, androblastoma, endometrium hyperplasy, endometriosis, embryoma, fibrosarcoma, Kaposi's sarcoma, hemangioma, cavernous hemangioma, angioblastoma, retinoblastoma, astrocytoma, neurofibroma, oligodendroglioma, medulloblastoma, ganglioneuroblastoma, glioma, rhabdomyosarcoma, hamartoblastoma, osteogenic sarcoma, leiomyosarcoma, thyroid sarcoma, Wilms tumor and the like.

105. A method of preventing or treating tumors, comprising administering the antibody or the functional fragment thereof of any one of claims 63 to 101to a patient.

106. A method of preventing or treating tumors of claim 105, wherein the tumor is any one tumor selected from the group consisting of colon cancer, colorectal cancer, lung cancer, breast cancer, brain tumor, malignant melanoma, renal cell carcinoma, bladder cancer, leukemia, lymphomas, T cell lymphomas, multiple

myeloma, gastric cancer, pancreas cancer, cervical cancer, endometrial carcinoma, ovarian cancer, esophageal cancer, liver cancer, head and neck squamous cell carcinoma, cutaneous cancer, urinary tract carcinoma, prostate cancer, choriocarcinoma, pharyngeal cancer, laryngeal cancer, thecomatosis, androblastoma, endometrium hyperplasy, endometriosis, embryoma, fibrosarcoma, Kaposi's sarcoma, hemangioma, cavernous hemangioma, angioblastoma, retinoblastoma, astrocytoma, neurofibroma, oligodendroglioma, medulloblastoma, ganglioneuroblastoma, glioma, rhabdomyosarcoma, hamartoblastoma, osteogenic sarcoma, leiomyosarcoma, thyroid sarcoma, Wilms tumor and the like.

107. A method of inducing apoptosis in carcinoma cells expressing TRAIL-R independently of exogenous factors, which comprises contacting an antibody or a functional fragment threreof of any one of claims 63 to 101 with carcinoma cells expressing TRAIL-R.

108. A method of producing an antibody or a functional fragment thereof of any one of claims 63 to 101, which comprisies,

(i) a step of immunizing an animal with TRAIL-R or a fragment thereof having the antigenicity, cells expressing the TRAIL-R or a fragment thereof having the antigenicity, or a DNA containing the gene encoding all or a part of the extracellular domain of TRAIL-R,

(ii) a step of obtaining antibodies from the animal,

(iii) a step of evaluating the activity of the antibodies to induce apoptosis in carcinoma cells expressing TRAIL-R independently of exogenous factors,

(iv) a step of separating a monomer antibody from the antibody,

(v) a step of evaluating the activity to induce apoptosis of the said monomer antibody, and

(vi) a step of selecting a monomer antibody having the activity to induce apoptosis.

ABSTRACT

Anti-TRAIL-R1 and R2 antibodies or functional fragments thereof, having at least one property selected from the following (a) to (c) of:

(a) having activity to induce apoptosis in carcinoma cells expressing TRAIL-R1 and/or TRAIL-R2;

(b) not having effect on normal human cells expressing TRAIL-R1 and/or TRAIL-R2; and

(c) not inducing human hepatocyte toxicity,

and an anti- TRAIL-R1 and R2 antibodies or functional fragments thereof, having the following properties: having activity to induce apoptosis in carcinoma cells independently of exogenous factors and as a monomer of an antibody.